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(54) Anti-Fas recombinant antibodies and DNA therefor

(57) A recombinant protein comprises at least one variable region corresponding to an immunoglobulin variable region specific for human Fas and has substan-

tially no more immunogenicity in a human than a human antibody, and is useful in the treatment of autoimmune diseases, especially rheumatism.

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suitably an expression vector. It may also be associated with any other suitable sequences, such as leader sequences or sequences for the expression of the encoded protein in the form of a fusion protein, for example.

The present invention further envisages a host cell transformed with a vector as defined above, and a system for the expression of a protein of the invention comprising such a host cell transformed with one or more expression vectors containing the above DNA. The protein of the invention may be obtained from such systems, after cultivation of the system, by standard techniques.

Certain preferred aspects and embodiments of the present invention now follow:

A genetically engineered immunoglobulin protein which specifically binds to human Fas, wherein the immunoglobulin protein consists essentially of an H chain subunit and an L chain subunit, the H chain subunit comprising an amino acid sequence represented by the following general formula (I):



wherein FRH<sub>1</sub> represents an amino acid sequence comprising 13 to 30 amino acid residues, CDRH<sub>1</sub> represents the amino acid sequence of SEQ ID NO. 1, FRH<sub>2</sub> represents an amino acid sequence comprising 14 amino acid residues, CDRH<sub>2</sub> represents the amino acid sequence of SEQ ID NO. 2, FRH<sub>3</sub> represents an amino acid sequence comprising 32 amino acid residues, CDRH<sub>3</sub> represents the amino acid sequence of SEQ ID NO. 3, and FRH<sub>4</sub> represents an amino acid sequence comprising 11 amino acid residues, each of the amino acid sequences being linked to the next via a peptide bond;

the L chain subunit comprising an amino acid sequence represented by the following general formula (II):



wherein FRL<sub>1</sub> represents an amino acid sequence comprising 23 amino acid residues, CDRL<sub>1</sub> represents the amino acid sequence of SEQ ID NO. 4, FRL<sub>2</sub> represents an amino acid sequence comprising 15 amino acid residues, CDRL<sub>2</sub> represents the amino acid sequence of SEQ ID NO. 5, FRL<sub>3</sub> represents an amino acid sequence comprising 32 amino acid residues, CDRL<sub>3</sub> represents the amino acid sequence of SEQ ID NO. 6, and FRL<sub>4</sub> represents an amino acid sequence comprising 10 amino acid residues, each of the amino acid sequences being linked to the next via a peptide bond.

The above protein is preferred, when the H chain subunit comprises the amino acid sequence represented by amino acids No. 1 to 116 in SEQ ID NO. 8, and/or wherein the L chain subunit comprises the amino acid sequence represented by amino acids No. 1 to 112 in SEQ ID NO. 10.

The above protein is more preferred when the H chain subunit comprises the amino acid sequence represented by amino acids No. 1 to 571 in SEQ ID NO. 8, and/or the L chain subunit comprises the amino acid sequence represented by amino acids No. 1 to 219 in SEQ ID NO. 10.

The invention further provides DNA encoding an H chain subunit comprising a peptide of formula (I) as defined above. We prefer such DNA when it comprises the nucleotide sequence represented by nucleotides No. 58 to 405 in SEQ ID NO. 7.

Also preferred is DNA which hybridises with such DNA, the sense strand encoding an immunoglobulin H chain subunit capable of specifically binding human Fas together with an L chain subunit comprising an amino acid sequence of general formula (II). It is preferred that such DNA is able to hybridise at 60 to 70°C and in 6 x SSC.

DNA coding for an L chain subunit comprising an amino acid sequence represented by general formula (II) is also preferred, especially where it comprises the nucleotide sequence represented by nucleotides No. 58 to 393 in SEQ ID NO. 9. DNA which hybridises such DNA and whose corresponding sense strand encodes an immunoglobulin L chain subunit which specifically binds to human Fas antigen together with an H chain subunit as defined above which comprises an amino acid sequence of general formula (I) is also preferred, especially where hybridisation is at 60 to 70°C in 6 x SSC.

Further preferred is a recombinant DNA vector containing any of the DNA described above, particularly recombinant DNA vectors pCR3-H123 and pCR3-L103, cells transformed with such vectors and especially *E. coli* pCR3-H123 (FERM BP-5247) and *E. coli* pCR3-L103 (FERM BP-5248).

A preferred method for producing an immunoglobulin protein which specifically recognises human Fas antigen comprises:

culturing a cell transformed by a DNA vector described above under conditions which enable expression of DNA encoding the immunoglobulin H chain or L chain subunit contained in the vector, and recovering the immunoglobulin protein from the culture.

herein may be cDNA synthesised by a reverse transcriptase reaction using mRNA obtained from a hybridoma producing anti-human Fas antibody, such as that which expresses CH11. The DNA fragment thus synthesised may either be directly integrated into a plasmid vector, such as by using a commercial kit, or may be labelled with, for example,  $^{32}\text{P}$ ,  $^{35}\text{S}$  or biotin, and then used as a probe for colony hybridisation or plaque hybridisation to obtain the desired clone.

5 Monoclonal antibody CH11 is an immunoglobulin M ("IgM") molecule, a complex comprising five subunits each of the H ( $\mu$  chain) and L chains, and one J chain. Thus, in order to elucidate partial amino acid sequences for the subunits, the subunits must be separated, and this can be done using any suitable technique, such as electrophoresis, column chromatography, etc. well known to those skilled in the art. Once the subunits have been separated, they can be sequenced, such as by the use of an automatic protein sequencer (for example, PPSQ-10 of Shimadzu), in order to determine the amino acid sequence of at least the N-terminal of each subunit. Oligonucleotides/primers can then be produced using this knowledge.

10 Harvesting of DNA encoding each subunit of anti-human Fas monoclonal antibody from the appropriate transformants obtained above may be performed by well known techniques, such as those described by Maniatis, T., et al. [in "Molecular Cloning A Laboratory Manual" Cold Spring Harbor Laboratory, NY, (1982)]. For example, the region of DNA 15 coding for the desired subunit may be excised from plasmid DNA after separating the fraction corresponding to the vector DNA from a transformant which has been determined to possess the necessary plasmid.

15 *E. coli* DH5 $\alpha$  has been transformed with plasmids containing DNA encoding the heavy, light and J chains of CH11, prepared as described above, and the resulting three transformants (designated *E. coli*pCR3-H123, *E. coli*pCR3-L103 and *E. coli* pCR3-J1123, respectively) have been deposited in accordance with the terms of the Budapest Treaty on 20 the Deposition of Microorganisms at the Research Institute of Life Science Technology of the Agency of Industrial Science and Technology on February 28, 1996, and have been allocated deposit Nos. FERM BP-5427, FERM BP-5428 and FERM BP-5429, respectively. *E. coli* DH5 $\alpha$  containing these plasmids may be cultivated in a directly comparable manner to *E. coli*DH5 $\alpha$  not possessing these plasmids. All deposited strains may be selected by their resistance 25 to ampicillin. The DNA of the present invention, therefore, may be obtained using these deposits. This can be done, for example, either by cultivating the deposits and isolating the plasmids, or by using the polymerase chain reaction 30 (PCR) using the plasmids as templates.

35 Wherever appropriate, DNA sequences may be sequenced in accordance by various well known methods in the art including, for example, the Maxam-Gilbert chemical modification technique [c.f. Maxam, A. M. and Gilbert, W. (1980) in "Methods in Enzymology" 65, 499-276] and the dideoxy chain termination method using M13 phage [c.f. Messing, J. and Vieira, J. (1982), Gene, 19, 269-276]. In recent years, a further method for sequencing DNA has gained wide acceptance, and involves the use of a fluorogenic dye in place of the conventional radioisotope in the dideoxy method. The whole process is computerised, including the reading of the nucleotide sequence after electrophoresis. Suitable machinery for the process is, for example, the Perkin-Elmer Sequence robot "CATALYST 800" and the Perkin-Elmer model 373A DNA Sequencer. The use of this technique renders the determination of DNA nucleotide sequences both efficient and safe.

Accordingly, from the thus determined nucleotide sequences of the DNA encoding the H and L chains of CH11, in conjunction with the sequence data for the N-termini of the H and L chains, it was possible to determine the entire amino acid sequence of the H and L chains of CH11.

Framework regions (FR's) are present in the variable regions of the H and L chains of immunoglobulins and serve 40 to flank the variable region and to separate the CDR's. Accordingly, there is one more FR compared to the number of CDR's in each variable region. The framework regions also contribute to the three-dimensional structure of the variable region, thereby assisting in antigenic binding by stabilising the tertiary structure of the region. By way of illustration of the terminology applied to each FR and CDR in the variable region, FRH<sub>1</sub> is the framework region at the N-terminal end of the variable region of the H chain. Working towards the C-terminal end, there is, respectively, CDRH<sub>1</sub>, FRH<sub>2</sub>, CDRH<sub>2</sub>, FRH<sub>3</sub>, CDRH<sub>3</sub> and, finally, FRH<sub>4</sub>. A similar nomenclature applies to the L chain, so that the first framework 45 region is designated FRL<sub>1</sub>, etc.

Both immunoglobulin H and L chains comprise a variable region and a constant region. The variable region is composed of CDR's and, as described above, both the H chain and the L chain have three CDR's interconnected and flanked by four framework regions. The amino acid sequences of the constant regions are the same, irrespective of the antigen recognised, and this is true separately for the H and L chains, provided that the immunoglobulin class is the same. By contrast, the amino acid sequence of the variable region, particularly the CDR's, is peculiar to each antibody. However, according to a comparative study on amino acid sequences of a number of antibodies, both the location of the CDR's and the length of the framework sequences connecting them are virtually constant among antibody subunits of the same subgroup [Kabat, E. A., et al. (1991), in "Sequence of Proteins of Immunological Interest Vol. II": U.S. Department of Health and Human Services]. With this knowledge, therefore, it was possible to determine the location of the CDR's, framework regions and the constant region of each of the H and L chains of CH11 by comparison of the amino acid sequences we had determined with the known amino acid sequences.

It will be appreciated that the framework regions are important for separating the CDR's and help in the steric

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Anti-Fas recombinant antibodies - plasmid expression in Escherichia coli,  
for use in rheumatoid arthritis therapy  
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CORPORATE SOURCE: Tokyo, Japan.  
PATENT ASSIGNEE: Sankyo 1997  
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LANGUAGE: English  
ABSTRACT: A new recombinant protein containing at least one region corresponding to an immunoglobulin (Ig) variable region, which enables the protein to recognize and specifically bind to a human Fas antigen, has no more immunogenicity in a human patient than a human antibody. The variable regions may be derived from a monoclonal antibody. Also claimed is a genetically engineered Ig protein which specifically binds to human Fas, RNA and DNA encoding the proteins, and humanized antibodies. The DNA may be contained on a vector, preferably plasmid pCR3-H123 and plasmid pCR3-L103, and used to transform a host cell, preferably Escherichia coli. The protein may be used in the treatment of an autoimmune disease, preferably rheumatoid arthritis. E. coli pCR3-H123 (FERM %BP%-%5247%) and E. coli pCR3-L103 (FERM BP-5248) are specifically claimed. (72pp)  
DESCRIPTORS: Fas recombinant humanized antibody prep., monoclonal antibody antibody engineering, plasmid pCR3-H123, plasmid pCR3-L103 expression in Escherichia coli, appl. rheumatoid arthritis therapy bacterium vector cloning (Vol.16, No.26)  
SECTION: PHARMACEUTICALS-Antibodies; GENETIC ENGINEERING AND FERMENTATION-